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Interest to Both United States and Royal Thai Government

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FOREWORD

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LTG Pucha Singharaj 10 Nov 97
PI - Signature Date

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I. INTRODUCTION

The Armed Forces Research Institute of Medical Sciences (AFRIMS) conducts research into infectious diseases with both military and public health relevance to both the United States and Royal Thai Governments. Studies leading to the prevention of HIV infections are of primary importance to the Royal Thai Army. In addition, malaria, dengue, hepatitis, Japanese encephalitis, scrub typhus, and infectious diarrhea are all areas in which the RTA have major interest.

Infection with the human immunodeficiency virus, type 1 (HIV-1), which causes the acquired immunodeficiency syndrome (AIDS), is pandemic. Current estimates indicate that at least 23 million people were infected as of the end of 1996, with a projected 30-40 million by the year 2000. More than 90% of infections exist in the developing world. The epidemic is currently exploding in South and Southeast Asia with more than 5 million infections estimated at the end of 1996, most of which have occurred in the past 6 years.

Efforts to prevent infection with HIV-1 are currently limited to education and behavioral change, including the use of "safer" sex measures such as condoms and limitation of sexual activities to monogamous relationships with monogamous partners. These measures have so far proved to have limited effectiveness. Vaccines for the prevention of HIV-1 disease and transmission have been under development for several years with testing beginning in the United States in both seronegative and seropositive patients in 1989 and 1990.

In 1990, researchers in the Department of Defense (DOD), among others, recognized the emerging HIV-1 epidemic in Thailand which had first become apparent in 1989 in intravenous drug users (IUDs). An agreement was made with the Royal Thai Army Medical Component (RTAMC) at the Armed Forces Research Institute of Medical Sciences (AFRIMS) to embark on a program of preparation for eventual field-efficacy evaluation of an appropriate vaccine candidate(s) for the prevention of HIV-1 disease and transmission.

Since 1991, The US Army Medical Component (USAMC) and the RTAMC at AFRIMS have conducted descriptive epidemiological studies of prevalent and incident infection with HIV-1 in Royal Thai Army conscripts, thereby contributing critical data to the high level characterization of the HIV-1 epidemic in Thailand. In January 1993, AFRIMS opened a Joint Clinical Research Center (JCRC) for the conduct of Phase I/II (safety & immunogenicity) trials of vaccine candidates in Bangkok. Since June of 1993, the HIV-1 research collaboration at AFRIMS has embarked on a program of cohort development to identify and prepare a population for eventual participation in the efficacy evaluation of an appropriate HIV-1 vaccine candidate.

II. BODY

1. General

Efforts made under the Cooperative Agreement during FY97 are focused in four general areas: 1) natural history of HIV infection in Thais; 2) characterization of potential cohorts for efficacy trials of a preventative vaccine; 3) phase I/II vaccine trials of candidate HIV vaccines; and 4) surveillance of HIV epidemic in Thailand.

2. Natural History Study

a. Introduction:

Understanding the natural history of HIV-1 infection is essential to planning for a phase III vaccine trial. There are many possible outcomes in the vaccinated subject who subsequently becomes exposed to HIV-1. In the best case scenario, HIV-1 vaccines may prevent infection (sterilizing immunity). However, protective vaccines (e.g. live attenuated polio vaccine) are thought to provide their clinical benefit through limiting (but not preventing) virus replication after challenge. Hence, although the induction of sterilizing immunity may be the ideal outcome in an HIV vaccine study, a product which induces an immune response which modifies viral replication, disease progression, or subsequent transmission is the more likely outcome.

Conceivably, vaccinees who are subsequently exposed to HIV-1 may demonstrate a booster effect of the immune response without infection, transient abortive infection, low grade controlled infection with a low viral load, unchanged symptoms of infection and viral load or, in the worst case, infection with higher than expected viral load, more severe symptoms and accelerated disease.

b. Study objectives:

(1) To characterize viral, immune regulatory and clinical sequelae in recently HIV-1 infected Thai men, during the first three years post-infection. These data may form the basis of efficacy endpoints in future prophylactic vaccine trials in Thailand;

(2) To characterize (genetically and serologically) circulating HIV-1 from recently infected Thai's. These data may form the basis for selection of vaccine strain prototypes for use in development of Thai-specific vaccine constructs; and

(3) To assess virus-specific and immune regulatory correlates of HIV infection/disease progression.

c. Methods:

Study population

This protocol contains plans for study of three groups of subjects: a prospective study of seroincident cases, a cross-sectional study of prospective cases and an evaluation of uninfected persons. The first groups are followed in order to document the natural history of infection during the first few years after infection. The second study is a cross-sectional look at prevalent HIV-1 patients representing the full range of HIV disease in Thailand. The third group provides data on normal values for the Thai population and serves as a control group for the other two populations studied.

Seroincident HIV-infected subjects

Persons with incident HIV infections from cohort studies in Thailand are recruited for this study. If willing, they sign a consent form to take part in the study. At that time they donate 50 ml of blood. The subjects also receive a physical examination and a brief questionnaire requesting information about their risk behaviors and recent medical history. The seroincident subjects are asked to return every 6 months for three years.

Seroprevalent HIV-infected subjects

HIV-infected subjects who enroll in this study are referred to the AFRIMS clinic from local physicians collaborating in the study.

Subjects without HIV-1 infection

Uninfected subjects in the study include Royal Thai Army personnel and staff who work at AFRIMS.

Laboratory methods

At the time of enrollment and at follow-up visits, a complete blood count (CBC) and lymphocyte immunophenotyping is done on all subjects. Diagnostic PCR is also conducted on PBMCs from seroincident and seroprevalent cases. Cells, plasma and sera are archived from each subject for future testing. Other testing, described below, will be done on a selected basis.

CBC and lymphocyte phenotyping

CBC and differential are measured using the Coulter MaxM counter. Lymphocyte immunophenotyping is performed using dual fluorescent staining and analyzed on the FACScan using Simulset software at AFRIMS.

PCR Subtyping

PBMC-derived DNA is used for PCR-based genotyping. HIV-1 subtypes are differentiated by nested PCR using primers in the gp 41 *env* region. Second round primers differentiated clades B and E, with the amplification of a 287 bp product.

d. Results

Study enrollment

Incident cases	108
Prevalent case	533
Seronegative case	108

Table 1 - Study population demographics

	Incident cases* <i>n</i> =108		Uninfected <i>n</i> =108		Prevalent+ <i>n</i> = 533	
	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)
Age						
20-29	99	(92)	77	(70)	236	(44)
30-39	7	(6)	23	(21)	234	(44)
40-49	1	(1)	7	(6)	62	(12)
Unk	1	(1)	1	(1)	1	(0)
Sex						
Male	94	(87)	81	(75)	348	(65)
Female	14	(13)	27	(25)	185	(35)

*includes subjects tested, who were enrolled in other prospective studies

+does not include prevalent cases for whom CD4 counts have been provided as a "service" to the Phramongkutklao Hospital HIV clinic

Table 2 - Summary of incident cases (n=108)

	n	(%)
Subtype (n=73)		
E	67	(62)
B	3	(3)
UN	38	(35)
Estimated year of seroconversion		
1992	29	(28)
1993	50	(48)
1994	9	(9)
1995	11	(10)
1996	5	(5)
1997	1	(1)
Number of follow-up visits		
1	66	(61)
2	22	(20)
≥3	20	(19)

Assay development and evaluation

The Departmental laboratories continue to provide clinical laboratory assays for the natural history study. Specimens for the development and establishment of laboratory assays by the Dept of Retrovirology are primarily provided by the natural history study. From January through October 1997, clinical labs have provided the following support for the natural history protocol:

<u>Laboratory</u>	<u>No. samples</u>	<u>No. assays</u>
CBC	251	502
Immunology (flow cytometry)	261	266

The transfer and development of assays for 1997 includes growth and titration of vaccinia constructs encoding HIV genes, EBV transformation of B lymphocytes (January), HIV-specific cytotoxic T lymphocyte (CTL) assays (May), natural killer cell assays (May) and the Chiron branched DNA assay (September). EBV transformation has been successful in over 85% of samples attempted. HIV-specific CTLs were demonstrated in the three natural history subjects tested at AFRIMS. Branched DNA assays have been satisfactorily conducted on 25 subjects enrolled in the natural history study (range <500 copies/ml-150,000 copies/ml).

The isolation of HIV from patient monocytes was undertaken as a research project with the aim of developing a potential vaccine candidate and/or reagent. To date, PBMC from 18 subjects have been assayed, which yielded one HIV subtype E isolate- NP 1517SG. Efforts are ongoing to further characterize this monocytic isolate. This represents the first HIV subtype E strain isolated directly from monocytes.

Optimization of virus isolation techniques included a comparison of isolation rates using coculture technique with seronegative donors from the US and Thailand. Results from PBMC collected from 10 HIV-infected subjects, including one HIV subtype B infection, imply that the target cell donor is a contributing factor in the rate of virus isolation, irrespective of the CD4 cell proportion. Peripheral blood mononuclear cells from 76 subjects were processed for virus isolation from January to October 1997, from which 26 isolates were obtained.

Plasma viral RNA quantitation

A group of approximately 120 subjects with adequate stored plasma collected in citrate, were selected for viral load testing using the new Roche primers in Rockville. This study provides pilot data on the correlation of CD4 counts and clinical status with viral load in men and women infected with HIV subtype E in Thailand.

Viral Diversity

Specimens from 12 seroconvertors from 1995-96 enrolled in the natural history were sent to Rockville for sequencing. Preliminary data indicates that diversity is intermediate between that of earlier Thailand prevalent subtype E cases and isolates from two AIDS subjects previously sequenced.

3. Cohort Studies

Cohort identification and characterization for Phase III trials is ongoing. This includes planning recruitment and follow-up mechanisms, determination of follow-up rates, HIV-1 incidence, behavior and STD rates in the population. Data collected from routine HIV-1 surveillance being conducted in the RTA, as well as several HIV-1 cohort studies, will provide information concerning cohorts which might be suitable for Phase III trials. Because the HIV epidemic in Thailand is dynamic and there are rapid changes occurring in the society, the process of identifying a suitable cohort has been challenging. Feasibility studies in two cohorts were begun in FY95 and continued through FY97. One of these is completed and the other will be completed in Q2FY97; in FY98 several new cohorts will be assessed.

Prevalence and incidence of HIV-1 infections among recruits in the Royal Thai Army at Prachuab Khiri Khan

a. Introduction:

Numerous studies have focused on the incidence and prevalence of HIV-1 infection among Royal Thai Army conscripts (Tahan Gahn). RTA conscript populations are socio-demographically homogeneous as relatively advantaged populations are excluded from conscription. Conscripts tend to be from non-municipal areas, engaged in agrarian occupations, possess a primary school education, and come from a Buddhist background. Those studies examining risk factors, interventions, or follow-up have focused on recruits in the Northern region where the epidemic has been most prominent.

Prachuab Khiri Khan is the southernmost province of the Central region in Thailand. Fort Thanarat, the major RTA installation in the province has conscripts from geographically diverse backgrounds. Conscripts who arrive for service in May generally come from the Central or Southern provinces, while those who arrive in May are drawn from the Northeast. Fort Thanarat was chosen because it had a large recruit population, increasing prevalence, predominantly non-deploying units (to simplify follow-up), and a single large hospital responsible for medical care. It's geographically diverse population also permitted exploration of regional differences in epidemiology and behavioral norms. The start date for this study was July 1995.

b. Study objectives:

- (1) Determine the prevalence and incidence of HIV-1 infection in recruits stationed at Fort Thanarat, Prachuab Khiri Khan Province, Thailand.
- (2) Assess the attitudes, behavior and follow-up patterns in these recruits.

c. Methods:

HIV-1 testing was done at baseline and then every 6 months. At each bleed, a questionnaire was administered to evaluate behavior and knowledge. Two different educational and behavioral intervention programs were implemented, using a non-randomized, quasi-experimental design. The incidence of HIV-1 in the recruits, overall and in the two intervention groups, will be determined, along with changes in knowledge and behavior over time. At the end of the follow-up period, subjects complete a questionnaire to assess attitudes towards participation in vaccine trials. As a service and incentive to the conscripts, hepatitis B virus immunization was offered, along with treatment of prevalent cases of syphilis. Regular follow-up and care is provided for

HIV seropositive participants in this study. The HIV care and behavioral interventions will be adapted by the Fort hospital and continued after the study is completed.

d. Results:

Number of subjects enrolled: 3839

Number of subjects to be enrolled: 3839

Analysis of baseline data has begun, with indications of cohort differences in risk for HIV prevalence. Implementation of the two interventions has been completed and the Fort hospital has assumed responsibility for continuing them. The RTA AIDS Committee has reviewed the behavioral intervention as a model for implementation at other posts. Follow-up data collection has been completed for two cohorts; the third will complete its final follow-up in Oct 97. Preparations are underway for analysis of incidence. Publication manuscripts are currently being written with regard to HIV-1 seroprevalence, patterns of baseline risk behavior, and the clinical care package that was offered to HIV-1 seropositive personnel at the fort. In addition, as the project begins to be closed out, transition of responsibility is occurring for medical treatment of asymptomatic HIV-positive recruits and preventive behavioral intervention activities to Fort Thanarat Hospital and to individual military units.

Due to the success for this program, there has been significant high level interest from both the U.S. Army and the Royal Thai Army, including:

- Received visits from LTG Ronald Blanck, US Army Surgeon General and COL Ernest Takafuji, Commander of WRAIR (now, Deputy Commander, US Army Research and Materiel Command).
- Received visit from Royal Thai Army AIDS Committee which studied the project for adaptation of behavioral intervention to other military installations.
- Presented results of preliminary incidence findings at Asia-Pacific AIDS Congress.
- Presented description of medical intervention program for asymptomatic HIV-seropositive recruits at Asia-Pacific AIDS Congress.
- Drafted article regarding behavioral intervention for submission to Soldiers (currently in revision).

Incidence of HIV-1 infection among persons attending STD clinics and anonymous test sites

a. Introduction:

This protocol studied the prevalence and incidence of HIV-1 infection in persons attending STD clinics in several areas of Thailand to determine whether this group would be a feasible cohort for HIV vaccine efficacy trials. The start date for the study was Sept 1995. The study was

completed in April 1997 and close out visits were done at each of the three sites. Data cleanup has been completed and analysis is ongoing.

b. Methods:

Subjects were enrolled from STD clinics and anonymous test centers at three sites, Bangkok, Chonburi, and Lampang. Participants were tested for HIV-1 at 4-month intervals for one year. Education and counseling were provided at each visit. At each bleed, a questionnaire was administered to evaluate behavior and knowledge. At the end of the follow-up period, subjects also completed a questionnaire to assess attitudes towards participation in vaccine trials.

c. Results:

Between September 1995 and February 1996, 1901 eligible persons were asked to participate in the study. Thirty percent of eligible men (371/1238) and 24% of women (161/663) agreed and were enrolled into the study. Among the 532 person who enrolled in the study, the HIV-1 seroprevalence was 3.4%. History of an ulcerative STD and lifetime CSW partners were associated with HIV-1 infection among men. There were no statistically significant risk factors identified for women. Follow-up at the 12-month study visit was 78%. There were 4 incident HIV infections; all among men.

Incidence of HIV-1 Infection Among Women Attending Family Planning Clinics in Rayong Province, Thailand

This protocol will determine the prevalence and incidence of HIV-1 infection in females attending three family planning clinics in Rayong and study the risk behavior and follow-up rates, as well as attitudes toward participation in future HIV-1 vaccine trials. This population represents a potential cohort for phase III HIV vaccine efficacy trials. The start date for the study is planned for 1 Dec 97, provided the logistics are in place to begin work. Contract finalization from the U.S. Embassy is pending.

Subjects will be enrolled from three family planning clinics in Rayong Province. Participants will be tested for HIV-1 at 6-month intervals for one year. Education and counseling will be provided. At each bleed, a questionnaire will be administered to evaluate risk behavior and HIV/AIDS knowledge. At the end of the follow-up period, subjects will complete a questionnaire to assess attitudes towards participation in future HIV-1 vaccine trials.

Approvals have been obtained from the RTA and Ministry of Public Health IRBs. Administrative approval has recently been given by the U.S. Army IRB, pending submission of a revised informed consent form which will be re-submitted Nov 97.

Proposal for Exploratory Collaboration on HIV-1 Project with Cambodian Ministry of Health

A proposal was made to the Cambodian Ministry of Health on 9 June 1997 to serotype positive HIV specimens from their Annual Sentinel Surveillance Program, perform QA/QC for the Annual Sentinel Surveillance Program, and to initiate an effort to explore HIV-1 incidence in a cohort of students over the next three years.

Proposal for a Community-Based Cohort

Preliminary investigations have been made in several communities to select an area that would be suitable for a community-based cohort. Visits were made to the provincial health offices in three provinces on or near the eastern seaboard. Several communities in Chonburi have been selected and preliminary discussions were held with local officials to plan resources needed for such an effort.

4. HIV-1 Vaccine Testing

a. Screening and evaluation of potential volunteers

The protocol was amended to include the two new TAVEG sites (Vaccine Trial Centre, Faculty of Tropical Medicine and Siriraj Hospital, both of Mahidol University) and to it more flexible as a screening tool for various vaccine protocols. Some examples: the age range was changed from 20 - 50 to be age 18 or older. The requirement for Thai nationality was removed, some of the specifics about lab assays and were made more general to allow flexibility in future studies, as were the descriptions of the sequence of procedures at each visit. A section was added to allow compensation to be paid for the last screening visit for those volunteers who are found to be eligible for the upcoming vaccine protocol.

b. Phase I/II trial of Chiron HIV SF2 rgp120 vaccine

This double-blind, randomized, Phase I/II study evaluated the safety/tolerability and immunogenicity of the human immunodeficiency virus SF2 gp120/MF59 vaccine (Chiron Vaccines) at the dose of 50 ug in two immunization schedules. The study population consisted of fifty-two HIV-1 seronegative, healthy Thai adults enrolled from the community, twenty-six at

AFRIMS in Bangkok and twenty-six in Chiang Mai. Each site had one drop out who was replaced, so a total of 54 volunteers were enrolled.

The final regular visit occurred 18 September 1996. Compliance was 100% for each visit at each site. Each subject was asked to return for three follow-up visits at 6-month intervals. The last one will occur on 29 November 97. The compliance rate (overall for 2 sites) was 96% of volunteers at first follow-up visit, 84% at the second, and 74% at the final (18-month) follow up visit.

Results to date indicate that the vaccine is safe. It induces no significant systemic toxicity or local reactogenicity; the safety profile of the vaccine in vaccinated Thais similar to that seen in volunteers who received this product in clinical trials in the United States. Binding and neutralizing antibodies were elicited, as were lymphoproliferative responses. These immune responses appeared greater in magnitude with the third dose at 6 rather than 4 months.

c. Phase I/II trial of Chiron HIV-1 Thai E rgp120 vaccine

During the FY97 period, preparations were completed for the first HIV vaccine trial with a candidate vaccine designed specifically for a developing world (a potential region of U.S. military deployment) HIV epidemic. The candidate vaccine, made by Chiron Vaccines, is a recombinant protein derived from the envelope glycoprotein 120 (rgp120) of the subtype E virus. This trial will determine safety, immunogenicity and optimal dose combination, and provide the basis for employment of this candidate vaccine in a phase III clinical efficacy trial to start in 2000-2001.

This phase I/II trial will be carried out in four sites in Thailand in nearly 400 HIV-negative, healthy volunteers. AFRIMS, in addition to being one of these sites, is the laboratory center for the three Bangkok sites. It is also the coordinator of this trial and has led the protocol development, multi-site staff training and site strengthening efforts. Having received approvals from all institutional IRBs and the Thai Ministry of Public Health, this rgp120(E) vaccine trial will begin its open-label phase in Nov 97 and the larger double-blind phase in Jan 98. Enrollment and immunization will each occur over 6-month periods, and follow-up will last 18 months.

d. Evaluation of HIV breakthrough infections

The protocol "Evaluation of HIV-1 Infection in Vaccine Trial Participants in Thailand" was approved in 1997. This study will define the immunologic, virologic and clinical outcomes of incident HIV-1 infection in persons who have participated in vaccine trials in Thailand. These observations will advance the understanding of correlates of immunity to HIV-1 infection,

vaccine design, viral targets for vaccine-induced immune responses, and HIV-mediated disease pathogenesis. They may also help define endpoints for Phase III studies.

Volunteers enrolled in trials of candidate HIV vaccines who have had a high risk exposure to HIV-1 and/or who are determined to be infected with HIV-1 after the first vaccine injection will be enrolled and followed prospectively. Volunteers with evidence of acute high risk exposure will be evaluated for 6 months. Follow-up will be continued if subjects have documented infection or continue to have high risk behavior. Laboratory/clinical evaluations include a physical exam, skin test, lymphocyte immunophenotyping, PCR and subtyping, CBC, viral load, CTL and lymphoproliferation studies.

5. Surveillance

Demographic information was collected on young men entering service with the Royal Thai Army nationwide and is merged with routine serologic HIV data collected by the RTA. The recruits were bled at entry into the RTA (every November and May) and sera were tested for HIV by ELISA (confirmed by Western blot). In 1996, serotyping of all HIV positive sera was initiated using a V3 peptide ELISA.

Data from this study is analyzed to evaluate trends in nationwide seroprevalence.

Trends in seroprevalence in the RTA

HIV-1 Seroprevalence (%) by Region of Service in the RTA and Year

Region	1990	1991	1992	1993	1994	1995	1996
Central	1.3	2.2	2.9	3.0	2.6	2.5	2.0
Bangkok	1.2	2.8	3.3	3.2	2.9	2.6	1.7
Northeast	0.9	1.8	2.4	2.6	2.5	1.6	1.6
North	6.1	6.5	7.5	7.3	5.0	3.4	3.3
South	1.6	2.2	2.7	2.8	2.2	2.1	2.1
Total	1.9	2.9	3.5	3.6	3.0	2.4	2.1

Serotyping

Over 95% of prevalent infections in 1996 were subtype E.

III. CONCLUSIONS

1. Natural History Study

The natural history study has been most useful as a tool for providing reagents for laboratory strengthening and development. It has also yielded potentially useful insights for further research. The natural history protocol has been revised to better address the needs of long-term follow-up for describing the natural history of HIV disease and defining endpoints for vaccine efficacy testing and to provide a mechanism for adequately following and evaluating vaccine subjects who develop HIV infection during vaccine trials.

2. Cohort Studies

Study of a civilian cohort (STD clinic attendees) was completed and study of a military cohort will complete in Q2 FY98. While these studies yielded valuable data, they also demonstrated that neither cohort was optimal as the potential study population for a phase III vaccine efficacy trial. Cohort development plans for FY98 include the study of women in MoPH family planning clinics and a proposed community-based cohort in Southeast Thailand.

The single-most important ingredient in successful cohort projects is a solid base of support and trust within the collaborating institutions and subject populations. The Royal Thai Ministry of Public Health and the network of government hospitals and clinics have been most cooperative in these efforts. The success of the cohort development process within MOPH facilities and with civilian subjects, has been based upon the establishment of working relationships with key individuals, including the Director, Department of Communicable Disease Control, the Director, Division of AIDS and with numerous MOPH officials at province, district and community levels. In the case of the RTA, success was based upon relationships built with hospital and base commanders, and support from the central command.

3. Phase I/II HIV Vaccine Trials

The first phase I/II trial of an HIV vaccine (rgp120) was completed with the vaccine found safe and immunogenic in Thais. Full analysis of that trial is ongoing. A large phase I/II trial of a subtype E HIV subunit vaccine (rgp120) has completed the approval process and will commence in Nov 97. The vaccine has been manufactured by Chiron Vaccines (USA); the trial will be carried out under a U.S. FDA IND with monitoring from Chiron and the Walter Reed Army Institute of Research. The trial, with nearly 400 subjects, will be held at three sites in Bangkok and one in Chiang Mai, with coordination from AFRIMS. This will be the first phase II trial of a non-B HIV vaccine in the world.

4. Surveillance

Active HIV surveillance of RTA conscripts has been invaluable. The data collected in this effort continues to provide one of the best windows to the dynamics of the HIV epidemic in Thailand. Serotyping has been initiated to better define the virological dynamics of the epidemic, particularly to assess potential intrusion of new subtypes and shifts in the current subtypes.

IV. Abstracts and Publications (FY97)

1. Karnasuta C, Pavanand K, Sattabongkot J, Chantakulkij S, Watt G: **Elimination of Plasmodium falciparum liver- stage parasites by antimalarial drugs.** Am J Trop Med Hyg 55(2):180 (abstract no. 247), 1996.
2. Chanbancherd P, Kasemsant S, Sukvit S, Klinputsorn R, Akapirat S, Brown A, de Souza M: **CD4+and CD8+ T- lymphocyte enumeration by fluorescent bead detection.** Thai AIDS J :191, 1996.
3. Kantipong C, Watt G, Jongsakul K, Chuenchitra C: **Infection with human immunodeficiency, virus does not influence the clinical severity of scrub typhus.** Clin Inf Dis 23:1168, 1996.
4. Gaywee J, Artenstein A, VanCott T, Trichavaroj R, Sukchamnong A, Amlee P, de Souza M, McCutchan F, Carr J, Markowitz L, Michael R, Nittayaphan S: **Correlation of Genetic and Serologic Approaches to HIV-1 Subtyping in Thailand.** J Acquir Immune Defic Synd Human Retrovirol 13:392, 1996.
5. Watt G, Chouriyagune C, Ruangweerayud R, al. e: **Scrub typhus infection poorly responsive to antibiotics in Northern Thailand.** Lancet 348:86, 1996.
6. Watt G, Win K: **Scrub Typhus Fever.** In Oxford Textbook of Medicine, 3rd edition, Oxford University Press :739, 1996.
7. Sirisopana N, Torugsa K, Mason C, Markowitz L, Jugsudee A, Supapongse T, Chuenchitra C, Michael R, Burke D, Singharaj P, Johnson A, McNeil J, McCutchan F, Carr J: **Correlates of HIV-1 Seropositivity Among Young Men in Thailand.** J Acquir Immune Defic Synd Human Retrovirol 11:492, 1996.
8. Stazzone A, Slaats S, Mortagy A, Kleinosky M, Diab A, Mourad A, Hebert A, Merrell B, RR W, Murphy J: **Frequency of Giardia and Cryptosporidium infections in Egyptian children as determined by convertional and immunofluorescence methods.** Pediatr-Infect-Dis-J 15(11):1044, 1996.
9. Karnasuta C, Watt G: **Enhanced detection of Plasmodium vivax liver stages by cytocentrifugation.** Parasitology Today 12:451, 1996.
10. Watt G: **Diagnosis of schistosomiasis.** Am J Trop Med Hyg :55(4), 1996.
11. Smith C, Brown A, Nakazawa S, Fujioka H, Aikawa M: **Multi-Organ Erythrocyte sequestration and ligand expression in rhesus monkeys infected with plasmodium coatneyi malaria.** AJTMH (55(4)):379, 1996.

12. Karnasuta C, Pongvongsa T, Jongsakul K, Na Nakorn A, Watt G: **Plasmodium ovale in Laos: first case report.** Southeast Asian J Trop Med Public Health , 1996, submitted.
13. de Souza M, Song M-S, Carr C, Sanders-Buell E, Shin Y-O, Artenstein A, Lee H-R, McCutchan F, Brown A: **HIV-1 Subtypes A and B Isolated from the Republic of Korea.** AIDS 11:389 , 1997.
14. Abu-Elyazeed R, Youssef F, Merrell B, El-Gama R, El-khoby T, Hassanein Y, Cummings C: **Praziquantel in the treatment of schistosoma mansoni infection: Comparison of 40 AND 60 MG/KG bodyweight regimens.** Am. J. Trop. Med. Hyg. 56(4):404, 1997.
15. Tanormklom S, Brown A, Jagodzinski L, VanCott T, Jugsudee A, Vahey M, Torugsa K, Supapongse T, Carr J, Mason C: **HIV Serotypes and RNA Levels in Thai Seroconverters.** J Infect Dis Antimicrobial Agents 14:143, 1997.
16. Michael N, Brown A, Voigt R, Frankel S, Mascola J, Brothers K, M L, Birx D, Cassol S: **Rapid Disease Progression without Seroconversion following Primary Human Immunodeficiency Virus Type 1 Infection-Evidence for Highly Susceptible Human Hosts.** J Infect Dis 175:1352, 1997.
17. Brown A, Malone J, Zhou S, Lane J, Hawkes C: **Human immunodeficiency virus ribonucleic acid (RNA) levels in US adults: a comparison based upon race and ethnicity.** J Infect Dis 176:794, 1997.
18. Brown A, McNeil J: **HIV vaccine development: a subtype E-specific strategy.** SE Asian J Trop Med Pub Health , 1997 in press.
19. Jenkins R, Patterson T: **HIV Locus of Control and Adaptation to Seropositivity.** J Applied Psychology , 1997 in press.

V. List of Personnel

PERSONNEL ASSIGNED UNDER CURRENT AGREEMENT

NO.	LOCATION	NAME	POSITION
1.	Data Room	Nucharee Thongsen	Chief of Data Room
2.	Data Room	Visut Lokpichat	Programmer
3.	Data Room	Wonlana Jaidee	Data Entry
4.	Data Room	Sithinan Suksuket	Data Entry
5.	Data Room	Suchat Thepsanan	Data Entry
6.	Data Room	Viroj Yamuthai	Data Entry
7.	Data Room	Supin Pankote	Data Entry
8.	AIP	Oranuch Adeeto	Special Project Technician
9.	AIP	Aporn Chitsunthornrat	Special Project Technician
10.	AIP	Kampol Puapuek	Research Assistant
11.	AIP	Sumonman Uttayamakul	Technician
12.	Lab CBC/CD4	Siriwat Ekapirat	Medical Technologist
13.	Lab CBC/CD4	Piraporn Uttchee	Medical Technologist
14.	Lab CBC/CD4	Vinai Kaneechit	Research Assistant
15.	Lab PCR	Athaya Ruangphueng	Technician
16.	JCRC	Suchat Chuangpho	Cleaner/Messenger
17.	HIV Office	Kritika Singharaj	Admin.Assistant
18.	HIV Office	Ploypailin Khlaimanee	Logistics Assistant
19.	HIV Office	Wareeporn Ongklang	PI. Secretary
20.	BAA	Yaowaluck Kitkangwal	BAA Secretary
21.	PKK	Naowarat Imlintharn	Nurse
22.	PKK	Somyos Panmai	Educator
23.	PKK	Petchpailin Khlaimanee	Secretary
24.	PKK	Thiti In-Ngam	Research Assistant
25.	PKK	Narongrid Pongpakdee	Research Assistant
26.	PKK	Somchai Puthasang	Driver